(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 June 2005 (23.06.2005)

PCT

(10) International Publication Number
WO 2005/055930 A3

(51) International Patent Classification[7]: A61K 35/14, 35/16, 38/00, C07K 1/00, 14/00, C12N 15/63, 15/85

(21) International Application Number: PCT/US2004/040234

(22) International Filing Date: 2 December 2004 (02.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/526,664 3 December 2003 (03.12.2003) US

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF ROCHESTER** [US/US]; 518 Hylan Building, Rochester, NY 14627 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FAY, Philip, J.** [US/US]; 43 Split Rock Road, Pittsford, NY 14534 (US). **WAKABAYASHI, Hironao** [JP/US]; 136 Greystone Lane #3, Rochester, NY 14618 (US).

(74) Agents: **MERKEL, Edwin, V.** et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 31051, Rochester, NY 14603-1051 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 18 August 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RECOMBINANT FACTOR VIII HAVING INCREASED SPECIFIC ACTIVITY

(57) Abstract: The present invention relates to recombinant factor VIII having a specific activity that is higher than that of the corresponding wild-type factor VIII. The present invention also relates to methods of making and using the recombinant factor VIII. The present invention also relates to an isolated nucleic acid molecule that encodes the recombinant factor VIII, as well as DNA expression systems and host cells containing the isolated nucleic acid molecule.

WO 2005/055930 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/40234

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 35/14, 35/16, 38/00; C07K 1/00, 14/00; C12N 15/63, 15/85
US CL : 530/380, 383; 435/69.1, 69.6, 320.1, 325; 5142, 802, 834; 536/23.1; 930/100
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/380, 383; 435/69.1, 69.6, 320.1, 325; 5142, 802, 834; 536/23.1; 930/100

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WAKABAYASHI et al. Residues 110-126 in the Factor VIII Heavy Chain Contain a Ca2+ Binding Site Required for Cofactor Activity. Blood, November 16, 2003, Vol. 102, No. 11, p. 542a, abstract 1988.	1-10, 13, 23-26, 32-42, 47 --- 1-11, 13-14, 16, 18-27, 29, 31-52
Y	US 5,859,204 A (LOLLAR) 12 January 1999 (12.01.99), abstract, columns 2-4, Col. 33, Table III, Col. 34, Table IV, Col. 40, Table V.	11, 19-22, and 48-52
Y	AMANO et al. Mutation at either Arg336 or Arg562 in Factor VIII is Insufficient for complete resistance to Activated Protein C (APC)-mediated inactivation: Implications for the APC resistance test. Thromb. Haemost. 1998, Vol. 79, pp. 557-563, especially abstract, p. 559, section bridging columns).	14, 27
Y	SWAROOP et al. Mutagenesis of Potential Immunoglobulin-binding Protein-binding site Enhances Secretion of Coagulation Factor VIII. J. Biol. Chem. 1997, Vol. 272, No. 39, pp. 24121-24124, especially abstract, p. 21123, fig. 1.	16, 29
Y, P	US 6,759,216 B1 (LOLLAR) 06 July 2004 (06.07.04), abstract, Col. 2, lines 54-67).	18, 31

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
01 June 2005 (01.06.2005)

Date of mailing of the international search report
23 JUN 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Holly Schnizer

Telephone No. (703 308-0196

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/40234

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WAKABAYAHI et al. Factor VIII:E113A Represents a High Specific Activity Factor VIII arising from a Single Point Mutation within the Ca2+ Binding Site. Blood, November 2004, Vol. 104, No. 11, p. 479a, abstract 1725.	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13
X, P --- Y, P	WAKABAYASHI et al. Residues 110-126 in the A1 domain of Factor VIII Contain a Ca2+ Binding Site Required for Cofactor Activity. J. Biol. Chem. March 2004, Vol. 279, No. 13, pp. 12677-12684.	1-10, 13, 23-26, 32-42, and 47 --- 1-11, 13-14, 16, 18-27, 29, 31-52
A	SARAFANOV et al. Cell Surface Heparan Sulfate Proteoglycans Participate in Factor VIII Catabolism Mediated by Low Density Lipoprotein Receptor-related Protein. J. Biol. Chem. April 2001, Vol. 276, No. 15, pp. 11970-11979.	12, 17, 30
A	LENTING et al. The sequence of Glu1811-Lys1818 of Human Blood Coagulation Factor VIII Comprises a Binding Site for Activated Factor IX. J. Biol. Chem. January 1996, Vol. 271, No. 4, pp. 1935-1940.	15 and 28

Continuation of B. FIELDS SEARCHED Item 3:
STN (Bioscience), EAST (all databases), sequence search, search terms: rfviii, factor viii, fviii, calcium binding, A1, Mn2+, Ca2+, hybrid, chimeric, inactivation cleavage site, factor IX binding, secretion, glycosylation recognition sequence.